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# On-line concentration of trace proteins by pH junctions in capillary electrophoresis with UV absorption detection

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**Abstract**

We report an on-line concentration approach based on pH junctions for the analysis of trace proteins under acidic conditions by capillary electrophoresis (CE) with UV absorption detection. Stacking is due to decreases in the electrophoretic mobilities of proteins when migrating from the sample zone to a relatively high-pH buffer filled in the capillary. Acidic buffers prepared from tris(hydroxymethyl)aminomethane (co-ions) and propanoic acid were suitable. With respect to speed, resolution, and stacking efficiency, it is appropriate to conduct the analysis of proteins under discontinuous conditions: pH 3.8 (inside the capillary), 2.8 (protein samples), and 3.3 (anodic reservoir). To minimize protein adsorption on the capillary wall, capillaries dynamically coated with single, double, and triple layers of polymers have been made and tested. Capillaries dynamically coated with three layers of neutral, cationic and neutral polymers in sequence were used to separate four proteins with good reproducibility. When using a 60-cm capillary, the peak height increased linearly with the injection volume up to 1.42-μl and peak profiles were sharp, indicating stacking of proteins. As a result, the limits of detection for lysozyme, myoglobin, carbonic anhydrase, and α-lactalbumin were 1.9, 3.2, 11.3 and 6.5 n*M*, respectively. Furthermore, this method has been applied to the analysis of about 1.31 and 0.66 μl of 5.00 and 0.20 μ*M* peptic and tryptic digests of β-casein, with results of detecting 26 and 12 peaks in 21 and 14 min, respectively.


*Keywords:* Sample stacking; Coated capillaries; Proteins; Poly(ethylene oxide)

## 1. Introduction

Capillary electrophoresis (CE) is processing toward maturity and becomes one of the most powerful analytical techniques [1]. For many years, many methods and instrumentation in CE have been developed and applied to analysis of a wide range of solutes, including small neutral molecules and ions, proteins, DNA and so on [2]. Although CE provides advantages over high-performance liquid chromatography, including rapidity, high-resolving power, and small amounts of samples and reagents required, it suffers from low concentration sensitivity because of a shorter optical path length, usually 50–100 μm, and a small injection volume (several nanoliters) [3]. To make CE more attractive, a number of sensitive detection techniques such as electrochemical methods, fluorescence, and mass spectrometry (MS) have been developed and tested with varying degrees of success [4,5]. However, they are either more sophisticated, expensive, selective, or not easily adapted to automation when compared with absorption detection.

To make CE as a more practical and powerful tool for trace analysis of proteins using absorption detection, developing concentration techniques is demanded. For example, analysis of proteins, such as

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human serum albumin (HSA) in urine [6], and amyloid and β-trace proteins in central nerve fluids, is important from a practical point of view [7]. The new techniques should also allow the analysis of hundreds of thousands of proteins, with concentrations ranging over six orders of magnitude, from biological samples because there is an urgent need to identify the patterns of protein expression, which is dependent on the stage of development of an organism and the organism's physiological state. Although CE could not play the role that two-dimensional gel electrophoresis combined with mass spectrometry does in proteomics [8], it has a large potential for determining the microheterogeneity of proteins because of its high resolving power and rapidity. However, time-consuming and costly preconcentration of certain proteins is generally carried out prior to CE analysis [9]. To circumvent these disadvantages, on-line concentration techniques have been developed, including isotachophoresis [10,11] and field amplification [12]. The concentration factors of these methods depend on the compositions of the discontinuous buffer electrolytes. Using membranes or hollow fibers has also been employed for the analysis of proteins, with good concentration results, but some shortages need to be overcome, including difficult automation and limits to certain proteins [13,14]. Recently, we have developed a technique allowing concentration of proteins and DNA prepared in either low- or high-conductivity media [15–17]. When DNA fragments or proteins migrating against electroosmotic flow (EOF) enter poly(ethylene oxide) (PEO), they slow down and stack at the boundary between the sample zone and PEO. As a result, more than 1500-fold sensitivity improvements in the protein analysis [16] and the analysis of up to 5-μl DNA samples have been demonstrated using laboratory-made CE systems with laser-induced fluorescence [17].

Adsorption of proteins on the capillary wall through Coulombic interactions, hydrogen bonding and/or hydrophobic patches leads to poor resolution and irreproducibility. Although using harsh conditions such as extremely high pH, low pH, or high salts to minimize adsorption has been demonstrated [18–20], protein denaturation, poor stacking efficiency, and/or Joule heating are problematic. To further overcome these problems, deactivated capillaries have commonly been used for protein analysis under mild conditions. The capillary wall could be either covalently bonded or dynamically modified with suitable chemicals or polymeric materials, while the latter provides the advantages of low cost and simplicity [21–35]. Common chemicals and materials used for dynamic modification of the capillary wall include amines, polycations, zwitterionic surfactants, PEO, hydroxycellulose derivatives, and linear polyacrylamide [27–33]. To prevent loss of resolution due to an incomplete coverage of the capillary wall with materials, multiple dynamic coating using positively and negatively charged polymers in between has been proposed [34,35]. Thus formed capillaries also allow switches of the direction of EOF, depending on the properties of the utmost layer of polymers. The stability of the capillary is excellent because Coulombic interactions take place between any two adjacent layers. Adsorption of proteins with opposed charges to that of the outmost capillary wall through Coulombic interactions could however be a problem.

The primary goal of this work is to optimize resolution, speed, and sensitivity for the analysis of proteins in CE with UV absorption. We developed a simple on-line concentration method for the analysis of proteins using deactivated capillaries. This method is based on the fact that the migration of proteins is solvent sensitive, depending on pH, ionic strength, electrolytes species, and buffer species [36]. We explored the effect of pH junctions and co-ions in the background electrolytes on the stacking efficiency. Using this method for peptide mapping was also demonstrated.

## 2. Materials and methods

### *2.1. Equipment*

A commercial electrophoresis instrument from Bio-Rad (BioFocus CE 2000, Hercules, CA, USA) was used. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 35 or 60 cm (360 μm O.D.×75 μm I.D.) At 4.5 cm from the outlet end, the polyimide coating was burned off to form the detection window. The absorbance of proteins was determined at 220 nm.

### 2.2. Chemicals

All chemicals for preparing background electrolytes, proteins and PEO were obtained from Sigma (St. Louis, MO, USA). Propanoate (PA) solutions were diluted from 1 *M* propanoic acid and adjusted with tris(hydroxymethyl)aminomethane (Tris) or NaOH to suitable pH values. Unless otherwise noted, *X* m*M* PA solution means that the total concentration of PA and propanoic acid is *X* m*M*. Carbonic anhydrase (CA), α-lactalbumin (α-Lac), myoglobin (Myo), lysozyme (Lys), ribonuclease A (Rib), trypsinogen (Try), β-lactoglobulin (β-Lac), and ovalbumin (Ova) were prepared in water and thus formed solutions were diluted to suitable concentrations with PA solutions prior to analysis. Pepsin and trypsin were separately prepared in 50 m*M* $NH_4HCO_3$ buffer, pH 8.0, and in 2% formic acid, pH 1.0, which then were mixed with β-casein (β-Cas), with a concentration ratio of 20:1. Tryptic digestion was conducted at 37 °C overnight and then was stopped by adding 0.1 *M* acetic acid, pH 4.0, while it was 1 h for peptic digestion.

### 2.3. Coating

Before coating, capillaries were rinsed with water for 30 min. The capillaries were flushed with either a neutral (0.5%) or ionic polymer solution (5%) for 15 min, and then equilibrated with the polymers at ambient overnight. Before processing in coating the second or third layer of the capillary wall with neutral or ionic polymers at room temperature for 10 min, the polymer solution inside the capillary was washed out with water. Fig. 1 shows thus formed capillaries that are called SLN, SLI, DLNI, DLIN, and TLNIN capillaries, wherein SL, DL, TL, N, and I mean single layer, double layers, triple layers, neutral, and ionic, respectively. I and N are arranged according to coating processes. For example, NIN means neutral, ionic, and neutral polymers were used to coat the capillary in sequence.

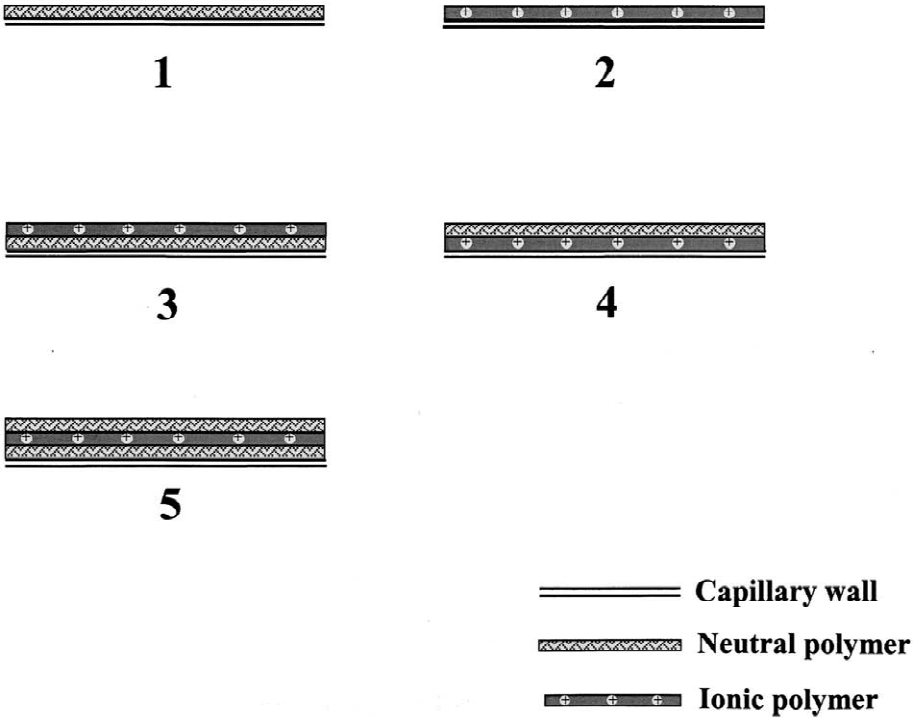


Fig. 1. Schematic of SLN (1), SLI (2), DLNI (3), DLIN (4) and TLNIN (5) capillaries.

### 2.4. On-line concentration and separation

Prior to pressure injection of the samples prepared in PA solution, pH 2.8, at low level, the capillary was hydrodynamically filled with PA solution (pH>2.8) at high level. The separation was conducted after the anode end of the capillary was immersed in a vial containing PA solution with different pH. Under these acidic conditions, proteins carrying positive charges migrated toward the cathode end. After each run, the capillary was re-equilibrated with polymer solution used to coat the outmost layer for 10 min before filling the capillary with PA solution.

## 3. Results and discussion

### 3.1. Dynamic coating

As the primary purpose of this study is to develop a method for performing on-line concentration of proteins under acidic conditions, an efforts was made to minimize adsorption of proteins on the capillary wall. Coulombic interaction is one of the most important contributors to protein adsorption when the charge of the outmost capillary wall is opposite to that of proteins. On the other hand, hydrogen bonding and hydrophobic patches are dominant when the outmost capillary wall is neutral and the charge density of proteins is low. We made and tested capillaries coated with one, two, and three layers of polymers, with either neutral or ionic polymers at the utmost layer, including SLN, SLI, DLNI, DLIN, and TLNIN capillaries. At pH<4.0, the ionic polymer is partially protonated and the four model proteins with p*I* values ranging from 4.7 to 11.0 are all positive. Thus, the main forces accounted for the interactions should be the last two. Table 1 shows that the relative standard deviation (RSD) values of the migration times for the four model proteins were less than 2.5%, despite using DLNI capillaries. Compared to SLI capillary, the positive charge density on the wall of DLNI capillary should be greater, because the ionic polymer did not directly interact with $Si–O^-$. Thus, stronger interactions with low-isoelectric point (low p*I*) proteins (α-Lac and CA) should account for high RSD. Our reasoning was supported by a very small EOF toward anode and greater migration times. The EOF mobility ($\mu_{eo}$) is less than $1\times10^{-5}$ $cm^2$ $V^{-1}$ $s^{-1}$ when using a DLNI capillary, which is larger than that (usually smaller than $3\cdot10^{-6}$ $cm^2$ $V^{-1}$ $s^{-1}$) of capillaries with neutral polymers at the utmost layer. In comparison, the analysis of acidic proteins (low p*I*) is reproducible when using DLIN and TLNIN capillaries. Although SLN and SLI capillaries provided good reproducibility, a relatively broad peak corresponding to CA was problematic. The peaks corresponding to Lys and Myo were sharper compared to those of α-Lac and CA when using the capillary coated with the cationic polymer. This result indicated that the interaction with acidic proteins was slightly stronger when the capillary wall was covered with a thin layer of polymers. Overall, we have shown that TLNIN capillaries provided the best performance in terms of stability and resolution. The RSD values of the migration times and peak heights for all proteins were <1.8 and <3.4%, respectively, in day-to-day runs for 3 consecutive days. Furthermore, several TLNIN capillaries were tested for more than 100 runs without a significant deterioration if washed with neutral polymer solutions after each run for 5 min. For this reason, we chose TLNIN capillaries for the following studies.

Table 1
Effect of coating procedures on the separation and quantitative reproducibility

| Capillary | Migration time (min) (RSD%)[a] | | | | | Peak height (mAu) (RSD%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lys | Myo | CA | α-Lac | System peak | Lys | Myo | CA | α-Lac |
| SLN | 7.81 (0.82) | 8.28 (0.81) | 9.65 (0.79) | 12.58 (0.89) | 12.97 (1.18) | 5.42 (3.21) | 5.86 (3.09) | 3.66 (2.65) | 4.79 (3.13) |
| SLI | 8.61 (0.64) | 9.06 (0.68) | 10.32 (0.82) | 12.85 (1.33) | 13.21 (1.32) | 5.62 (2.35) | 6.54 (2.43) | 3.27 (3.19) | 3.91 (2.85) |
| DLNI | 8.48 (3.05) | 9.04 (3.37) | 10.77 (4.03) | 14.82 (5.84) | 15.86 (6.52) | 5.06 (4.35) | 7.81 (4.80) | 2.54 (5.42) | 3.52 (6.12) |
| DLIN | 7.96 (2.13) | 8.39 (1.73) | 9.70 (0.97) | 12.15 (0.58) | 12.47 (0.89) | 5.18 (3.70) | 5.18 (2.85) | 2.68 (2.64) | 3.66 (2.35) |
| TLNIN | 7.83 (1.80) | 8.31 (1.80) | 9.59 (1.63) | 12.53 (1.36) | 13.24 (1.20) | 6.74 (3.40) | 8.50 (2.75) | 2.78 (2.50) | 3.81 (2.95) |

[a] $n=5$, and the sample was injected at the low level for 1 s.

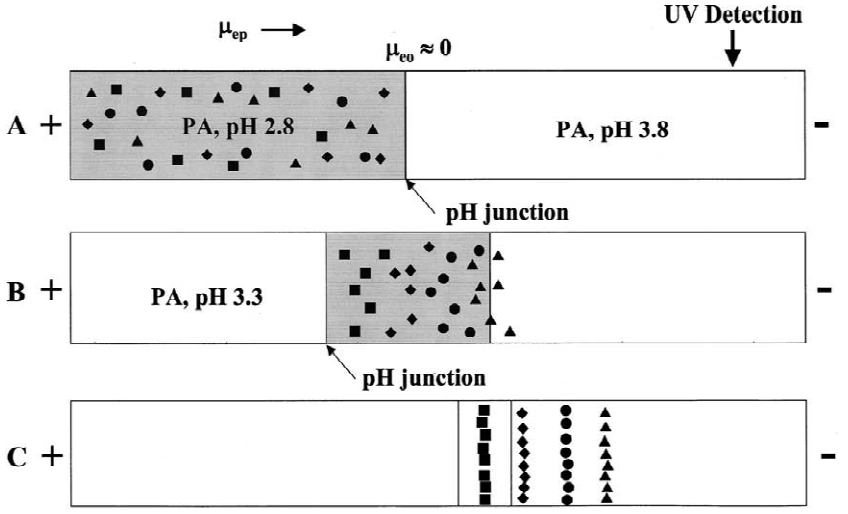


Fig. 2. Evolution of on-line concentration of trace proteins by pH junction. (A) After pressure injection of protein sample; (B) stacking of proteins; and (C) separation of protein based on the charge-to-mass ratio.

### 3.2. Stacking

Based on the changes in the electrophoretic mobility ($\mu_{ep}$) of proteins at different pH values, we developed an on-line concentration technique based on pH junction. At pH<4.0, the four model proteins are positive and migrate toward the cathode end. When migrating from low-pH sample zone to high-pH electrolytes, the net positively charges of proteins decrease. As a result, proteins slow down and stack at the boundary. To generate discontinuous conditions (pH junctions) inside the capillary, 200 m*M* PA solutions, pH 3.8, and 2.8, were used to fill the capillary and to prepare protein samples, respectively. To make sure that the four proteins carried positive charges, the capillary was placed in an anode vial containing 200 m*M* PA, pH 3.3, during the separation. A cartoon is given in Fig. 2 to show pH junctions in the system. After stacking, the analysis was carried out at pH 3.3 for optimum resolution. The use of PA as the background electrolyte provides advantages of low Joule heating generated at high electric field strengths and low absorption background at 220 nm. For example, the current generated at 500 V/cm was only 8.2 μA when the separation was carried out in 200 m*M* PA solution, pH 2.8, under isocratic conditions.

Using a 35-cm capillary, the four proteins injected at low level for 40 s were still resolved. The injection length and volume were about 17.2 cm and 760 nl, respectively. Table 2 shows good linearity between the peak height and injection volume, with

Table 2
On-line concentration and separation of four model proteins by pH junctions using a 35-cm capillary

| | Lys | Myo | CA | α-Lac |
|---|---|---|---|---|
| Linear regression[a] | $y = 0.023x - 1.28$ | $y = 0.029x - 1.72$ | $y = 0.004x - 0.19$ | $y = 0.015x - 0.74$ |
| Linear regression coefficient ($R^2$) | 0.996 | 0.997 | 0.942 | 0.992 |
| LOD (n*M*) ($S/N=3$)[b] | 3.1 | 5.0 | 25.2 | 10.3 |
| CF[b] | 48 | 69 | 40 | 30 |
| Migration time (min) (% RSD)[b,c] | 3.63 (0.61) | 3.79 (0.58) | 4.27 (0.64) | 5.27 (0.59) |

[a] $y$ is the peak height (mAU); $x$ is the injection volume (nl).
[b] Injection volume was 760 nl.
[c] $n=4$.

linear regression coefficients ($R^2$) greater than 0.99 except for CA (0.942), indicating that proteins stacked well. Owing to a relatively broader peak profile for CA mainly due to its microheterogeneity [37], the deviation of linearity was slightly greater. With increasing injection volumes, the migration times shortened since proteins migrated in a greater fraction of low pH zone. For example, the migration times for Lys and α-Lac shortened from 4.74 and 9.69 min to 3.63 and 5.27 min, respectively, when the injecting length was increased from 2.2 to 17.2 cm. Unfortunately, resolution decreased with increasing the injection length, with a limit of about 17.2 cm. With a maximum injection (four peaks were still resolved in baseline), the concentration factor (CF) values for the four proteins ranged from 29 to 70, with the limit of detection (LOD) values in the order of several tens n*M* at a signal-to-noise ($S/N$) ratio of 3. Herein CF was used to express the stacking efficiency, which is defined as the LOD obtained by conventional injection (1 s at the low pressure level) to that obtained for longer injection times (e.g. 40 s) at the same pressure level. The LOD is only slightly higher than that obtained using a very expensive argon laser at 275 nm without performing on-line concentration [38]. Although increasing injection volumes is useful to further improve sensitivity, it generally suffers from poor resolution. To overcome this shortage, a longer capillary was tested. By using a 60-cm capillary, the injection length was extended to about 32 cm, which corresponds to 1.42 μl. The LODs for Lys, Myo, CA, and α-Lac were 1.9, 3.2, 11.3 and 6.5 n*M*, respectively, and the sensitivity improvements were about 76, 107, 89 and 47-fold at the expense of a longer analysis time (<12 min).

### *3.3. pH dependence*

To exploit pH effect on stacking, we performed analyses using 200 m*M* PA solutions, pH 2.8, 3.3, 3.8 and 4.3, respectively, to fill the capillary and anode vials. Fig. 3A and B show that there were no sharp peaks (a bump) at pH 2.8 or 3.3 when injecting about 0.67 μl protein sample, indicating poor stacking because of small differences in pH and conductivity. The four main peaks were resolved as shown in Fig. 3C and D, while it took 20 min to complete the analysis at pH 4.3 and the stacking for

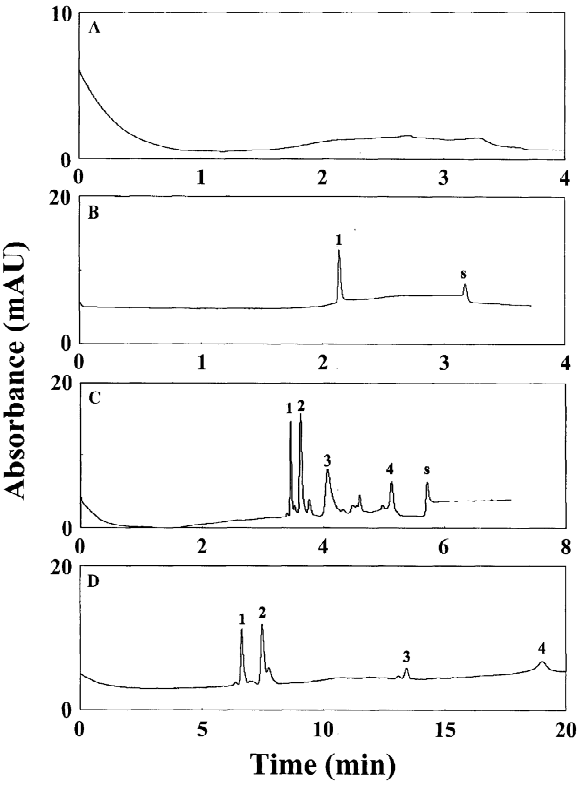


Fig. 3. Effect of pH on the separation of the four model proteins at 15 kV. The pH values for PA solutions in the anode reservoirs and the capillary were 2.8, 3.3, 3.8 and 4.3 in (A), (B), (C) and (D), respectively. Proteins were prepared in 200 m*M* PA, pH 2.8. The injection volume was about 0.67 μl. Peaks: 1=Lys (0.25 μ*M*); 2=Myo (0.5 μ*M*); 3=CA (0.5 μ*M*); 4=α-Lac (0.25 μ*M*); s=system peak. The capillary was 35 cm total length (30.5 cm effective length).

CA (p*I* 6.2) and α-Lac (p*I* 4.7) were not as effective as that at pH 3.8. Because hydrogen ions migrated much faster than proteins, the proteins close to the anode end slowed down before migrating at the interface in the cathode end, leading to poor stacking. This effect was more profound for the acidic proteins. Regarding the speed and stacking efficiency for the acidic proteins, the optimum condition was pH 3.8. However using a higher pH condition is suggested when high resolving power is needed. In comparison with the results shown in Fig. 3C and D, we concluded that pH did play a significant role in determining $\mu_{ep}$, thereby affecting stacking efficiency, speed and resolution.

To further evaluate pH effect, a short plug (about

0.4 cm) of 30 or 50 m*M* phosphate solution adjusted with NaOH to apparent pH 12.0 was applied prior to sample injection. Electropherograms depicted in Fig. 4 show slow separations in the presence of the plugs. In addition, the migration times increased while the stacking efficiency decreased with increasing the concentration of phosphate solution. This is mainly due to titration of hydrogen ions with greater concentrations of anions (phosphate and hydroxide ions). As a result, proteins had less positive charges and slowed down gradually before entering the PA in the cathode end, leading to loss of stacking (not shown). Owing to a relatively short length of phosphate solution compared to that of the sample plug (about 15.1 cm), proteins should still migrate under acidic conditions. This suggestion was supported by short migration times for the four proteins when compared to the result obtained at pH 4.3 (Fig. 3D). Apparently, it is promising for optimum resolution by applying a suitable plug of phosphate solution, with a support from a complete separation of eight proteins shown in Fig. 5. In the presence of a short plug of 50 m*M* phosphate, the separation of a 1.42 μl sample containing eight proteins was complete in 23 min.

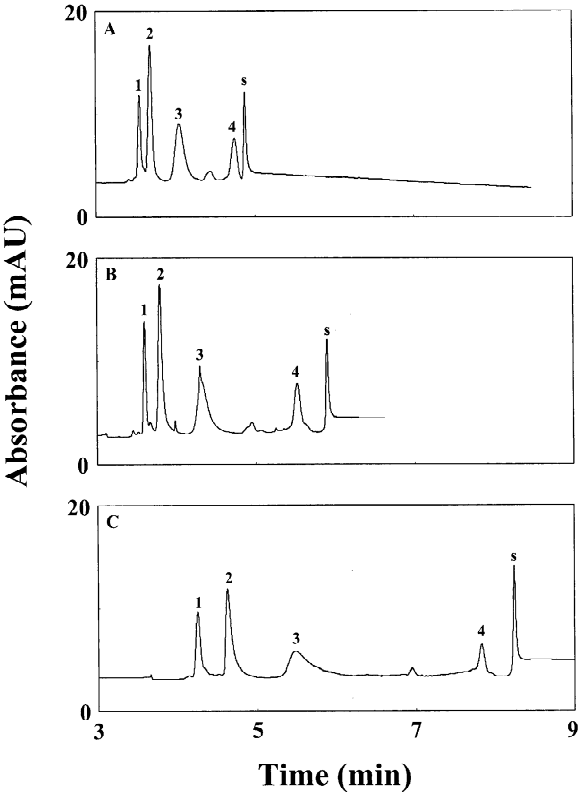


Fig. 4. Effect of 0.4-cm phosphate plug prior to sample injection on the separation of proteins. (A) No plug; (B,C) 30 and 50 m*M* phosphate, apparent pH 12.0, respectively. The anode vial contained 200 m*M* PA, pH 3.3. Proteins were prepared in 200 m*M* PA, pH 2.8. The injection volume was about 1.42 μl. Peaks: 1=Lys (0.25 μ*M*); 2=Myo (0.5 μ*M*); 3=CA (0.5 μ*M*); 4=α-Lac (0.25 μ*M*); s=system peak. Other conditions as in Fig. 3.

### *3.4. Effect of co-ion*

To test the effect of co-ions, we performed the analysis of proteins using PA solutions adjusted with Tris or NaOH. When the capillary was filled with PA (adjusted with NaOH), pH 3.3 and placed in PA, pH 3.8 during separation, CA and α-Lac (migration time was 3.9 min) were not resolved from the system peak (result not shown). In addition, the migration times for the four proteins were all shorter in the presence of sodium ions, indicating increases in the $\mu_{ep}$. This shows that the positively charged density of proteins increased upon interacting with sodium ions, which is in good agreement with a reported result [39]. On the other hand, a complete separation of the four proteins was achieved in 7 min in the case of using Tris to adjust the pH. One other advantage of using the solution containing Tris is that the stacking efficiency is greater (2-fold). With a smaller $\mu_{ep}$, Tris cations acted like a terminating ions in the system and thus affected the stacking efficiency [40]. The fact that the conductivities were different in the two different conditions indicated that the changes in the viscosity should slightly affect the separation results.

### *3.5. Peptide mapping*

Because the net charges and electrophoretic mobility of proteins are both dependent on ionic strength, the effect of salt on the analysis of peptic and tryptic digests must be explored. The salt impact on resolution, speed, and stacking efficiency is expected to be more profound when injecting large volumes of samples (0.76 μl). In the presence of 1 m*M* NaCl in the sample containing the four model proteins, the changes in the migration time and stacking efficiency were negligent (<1%). Although

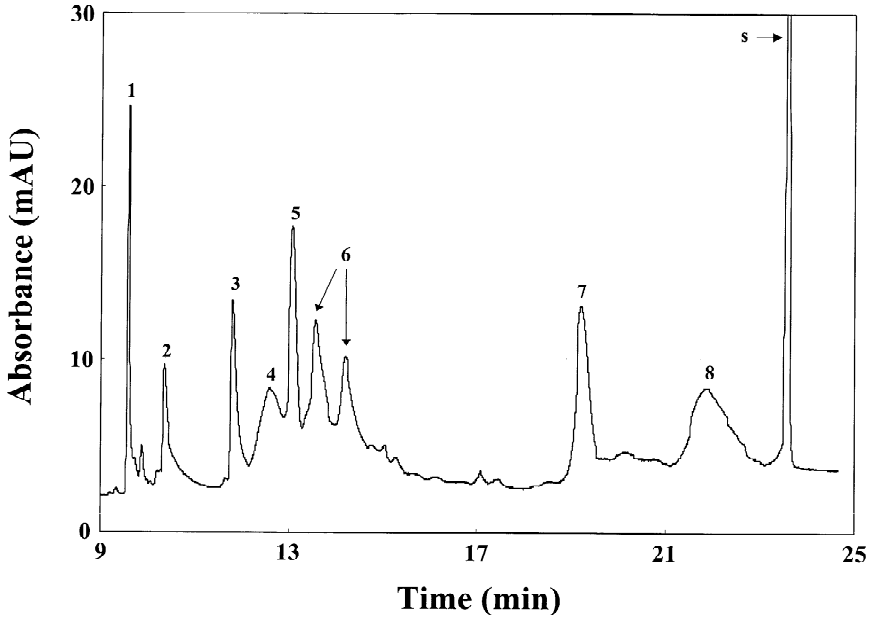


Fig. 5. On-line concentration of eight proteins in the presence of a short plug of 50 m*M* phosphate solution, apparent pH 12.0, at 20 kV. Peak: 1 = Lys; 2 = Myo; 3 = Rib; 4 = CA; 5 = Try; 6 = β-Lac; 7 = α-Lac; 8 = Ova; s = system peak. The concentrations of all proteins were 0.9 μ*M*. The capillary was 60 cm in total length (55.5 cm effective length). Other conditions as in Fig. 4.

the migration time for α-Lac increased to 13.5 min in the presence of 10 m*M* NaCl, the stacking was still effective supported by sharp peak profiles. In the presence of more than 20 m*M* NaCl, the analysis was failed, presumably because of Joule heating and small electrophoretic mobilities of proteins. These results suggested that matrix effect is significant using this proposed method. To minimize the salt effect, we separately diluted peptic and tryptic peptides of β-Cas with 200 m*M* PA solution, pH 2.8, based on the results shown above. When injecting the digests of β-Cas (0.2 or 5 μ*M*), 12 and 26 peaks were reproducibly detected in 14 and 21 min as shown in Fig. 6A and B, respectively. Although these peaks were not identified in this work, we expect that this would be possible if CE–MS were applied; this is not available in this laboratory. Because the two β-Cas samples were digested under different environments, the times for the system peaks (not shown) were different. From a practical point of view, this is one shortcoming of this method. Despite this, the concentration used in this study was about 2 orders of magnitude less than that used in a conventional method [41], showing the advantage of this proposed method for trace analysis of proteins.

## 4. Conclusions

Capillaries modified with multilayers of adjacent polymers with neutral and cationic charges have been fabricated and tested for protein analysis, with high resolving power, long lifetime, and reproducibility. Of these capillaries, TLNIN ones are best and have been used for on-line concentration of proteins under acidic conditions. When migrating from the sample zone to PA solution (relatively high pH), proteins slow down and stack. We have found that co-ions (Tris) play a significant role in determining stacking efficiency. When using a 60-cm capillary, the injecting volume was up to about 1.42 μl, leading to LOD values for Lys, Myo, CA, and α-Lac of 1.9, 3.2, 11.3 and 6.5 n*M*, respectively. This result demonstrates the suitability of this method for the analysis of trace proteins.

Although salts affect the stacking efficiency, this method has been used for the analysis of 0.2 μ*M*

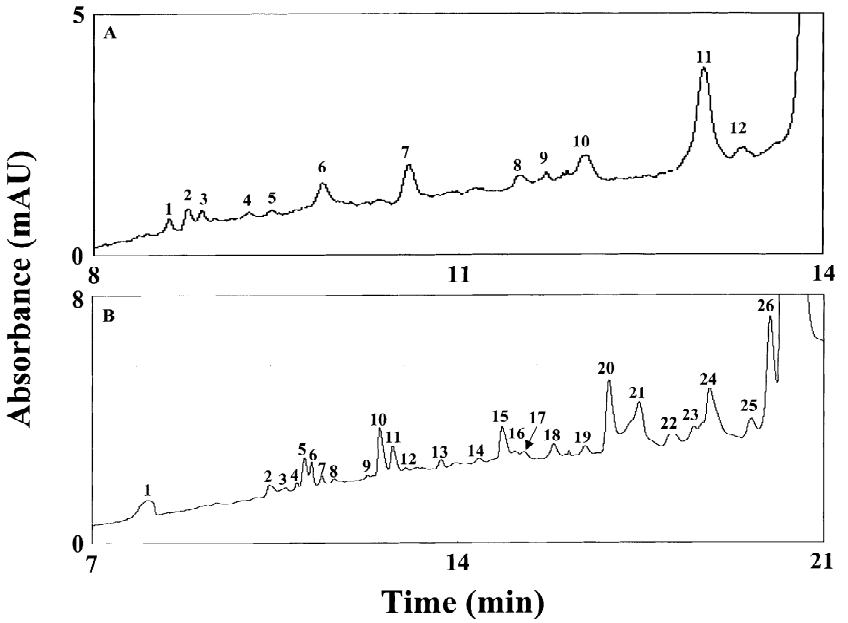


Fig. 6. Electropherograms of peptides from β-Cas at 20 kV. Peptic and tryptic digests in (A) and (B), respectively. The anode vial contained 200 m*M* PA, pH 3.3. Peptides were prepared in 200 m*M* PA solution, pH 2.8. The injection volumes were about 1.31 and 0.66 μl in (A) and (B), respectively. Peak numbers given in (A) and (B) without identification. Other conditions as in Fig. 5.

peptic digest of β-Cas prepared in 200 m*M* PA solution, pH 2,8. With good sensitivity, high-resolving power, simplicity, rapidity, reproducibility, it is our future goal to combine this technique with MS to identify trace proteins. In conjunction with immunoassay, this new approach will be applied to the analysis of important biological markers such as CA in a variety of biological samples. We will also extend this technique to the analysis of trace proteins labeled with suitable fluorophores using laser-induced fluorescence.

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